ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 3, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

FORMULATION AND EVALUATION OF ANTIAGING PHYTOSOMAL GEL

JULIE MARIAM JOSHUA, ATHIRA ANILKUMAR, VERJINA CU, DEEPA T VASUDEVAN, SARITHA A SURENDRAN*

Department of Pharmaceutics, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Cochin, Kerala, India.
Email: saritha19489@aims.amrita.edu

Received: 14 December 2017, Revised and Accepted: 29 January 2018

ABSTRACT

Objective: The aim of the present study was to prepare and evaluate antiaging phytosomal gel.

Method: For this purpose, tender coconut water, *Aloe vera* extract, grape seed extract, vitamin E, and jojoba oil were taken. The cytokinins present in coconut water show astounding effects in preventing premature aging. *A. vera* contains Vitamin E and C and phenol compounds which contribute to aging. Grape seed extract contains polyphenols mainly in the form of flavonoids which are highly potent antioxidants. It also possessed an antiaging vitamin known as oligomeric proanthocyanidins which prevented premature aging. Vitamin E was chosen because of its antioxidant properties. Jojoba oil was chosen because it had good moisturizing properties and it was similar to human skin oil, sebum. Conventional dosage forms such as creams and gels were prepared using these ingredients, but they showed less antiaging effect. Therefore, phytosome was prepared by binding herbal extracts to phosphatidylcholine (acts as a carrier and nourishes the skin). They had a phospholipid molecular structure which included a water-soluble head and two fat-soluble tails, and due to this dual solubility, it was better absorbed and so it was used for the treatment of skin disorders, antiaging, and skin carcinomas.

Result: Nine different formulations of the antiaging cream, gel, and phytosomal gel were prepared. Physicochemical parameters such as pH, viscosity, homogeneity, spreadability, and extrudability were determined. *In vitro* antioxidant studies were performed for the prepared antiaging cream, gel, and phytosomal gel. Among the three different formulations, phytosomal gel was chosen to be the best formulation to treat wrinkles and blemishes on the skin. When compared to the conventional dosage forms such as creams and gels, the phytosomal gel could release the herbal ingredients and showed better penetration into the skin. From the nine different formulations, F2 containing both tender coconut water and *A. vera* extract was chosen as the optimized formula. Optimization was done on the basis of *in vitro* antioxidant studies and physicochemical parameters. F2 acts as a potent free radical scavenger and inhibits oxidation by free radicals. 2,2-diphenylpicrylhydrazyl free radical scavenging assay was chosen as the best method in screening the antioxidant activity of the herbal extracts. The IC_{50} value of the prepared antiaging cream, gel, and phytosomal gel was found to be 70.5 µg/ml, 65.0 µg/ml, and 47.0 µg/ml, respectively. The lower the IC_{50} value, the highest the antioxidant activity. Thus, the antiaging phytosomal gel proved to show the highest antioxidant activity. The stability of the optimized formulation was carried out at two different temperatures, 30°C ± 2°C and 4°C ± 2°C, and the formulation was found to be stable at the end of 45 days.

Conclusion: From the present study, it can be concluded that the prepared antiaging phytosomal gel was safe, convenient, and efficient carrier to deliver the herbal extracts. It also showed better penetration into the skin. Hence, the desired antiaging property was obtained. Hence, the desired antiaging property was obtained and is used in skin care cosmetics.

Keywords: Antiaging, Phytosome, gel, Flavonoids, Herbal extracts.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i3.24257

INTRODUCTION

Aging is a very common phenomenon to all living beings. It is signified by progressive deterioration and degeneration of cells and organs. As far as human beings are concerned, aging is a complex phenomenon of physical, psychological, and social changes. According to biologists, aging is a sum of all changes that occur in a living being with the passage of time and lead to a decreasing ability to survive stress, to functional impairment, and finally to death [1,2].

The effect of aging on the world population does have an indirect effect on the worldwide medical care and health scenarios [3]. The United Nations have stated that the number of people worldwide of the age group 60 or above will be rising one in 10 to the current one in five ratios by 2050 and the ratio in developing nations and developed nations are going to double and triple, respectively. These demographic statistics suggest healthcare scenario to be more careful with the changes associated with aging and to concentrate on more antiaging therapies [4,5].

The theories involved in the aging process are mutation accumulation and antagonistic pleiotropy theory. The symptoms include atrophy, laxity, wrinkling, sagging, dryness, blemishes, and sparse gray hair. Being the largest organ of the body, in case of body weight and surface area, skin has the most profound and observable changes of aging [6].

Cosmeceutical is a term used to refer to all the combinations of cosmeceutics and pharmaceuticals. These are mostly the hybrid products of pharmaceutics and cosmetics. These are meant for improving the beauty and skin tone through natural ingredients [7,8]. Cosmeceuticals are the most mushrooming arena of natural personal care industry. However, the sad part of story is that a large percentage of these products do have a lot of carcinogenic agents such as diethanolamine and triethanolamine (TEA). Due to these side effects, researchers have now turned to the natural side of the field. Herbal cosmetics are now a fine area of interest for researchers.

The antioxidants such as alpha-tocopherol and ascorbic acid are used widely these days as they are safer and have less adverse effects than the synthetic antioxidants. The mechanism of antioxidants is the scavenging of the free radicals produced *in vivo* results in cell death and tissue damage. Thus, antioxidants are vital substances which

specifically protect the body from the damages caused by these reactive oxygen species [10,11].

Cytokines are a group of plant growth hormone, involved in cell growth and differentiation. These are also known as antiaging hormones as aging of plants is affected by these hormones. Coconut water was found to have antiaging properties. It was found that antioxidants and cytokines in coconut water have profound action against the aging phenomenon [12].

Antiaging cream reduces the wrinkles and blemishes on the skin. The advantage is that these have lesser penetrations and can be removed by sweating or a mild washing of the face. Gel is a semisolid preparation with two interpenetrating phases: A gelling phase and a liquid. The continuous liquid phase allows free diffusion of molecules through the skin. Gels are preferred to creams as they have less emollient effects and no pore clogging. There is also enhanced penetration of drugs [26,28].

Phytosomes are very recent introduction into herbal formulation as they are better absorbed and have higher bioavailability. The term phyto means "plant" while "some" means cell like. This are advanced forms of herbal formulation that contains bioactive phytoconstituents of herbal extract surrounded by a lipid. Phytosomes have a better pharmacokinetic and pharmacodynamic profile. In short, phytosomes are a bridge of connection between the conventional drug delivery system and the novel drug delivery system [30].

Advantages of phytosomes are increased bioavailability, efficient nutrient safety, and optimum entrapment efficiency. Phosphatidylchloride in phytosomes has a role both as a skin nourisher and also nourishes the skin. The phytosomes have better clinical benefits. The structural properties are also superior and are unique. Phytosomes also can cross cell membranes and enter cells. Being a complex between a natural products and natural phospholipid, there is the formation of hydrogen bond between the two compounds. With water, phytosomes assume a micelle shape. These are better absorbed, utilized, and have better results. It has higher bioavailability than conventional drug. There are increased pharmacokinetic and pharmacodynamic properties [32,33].

Phytosomes are used in liver diseases and heart diseases. It is also used as anti-inflammatory, lipolytic, vasokinetic, trophodermic, immunomodulatory, and antioxidant agents. Phytosomes are a patented technology developed by a leading manufacturer of drugs and nutraceuticals to incorporate standardized plant extracts or water-soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes called as phytosomes and so vastly improve their absorption and bioavailability [35].

MATERIALS AND METHODS

Materials

Tender coconut water was obtained from CDB lab, South Ernakulum. *Aloe vera* extract was obtained from Elixir Extracts, Nellad Kochi.

Methods

Formulation of O/W cream

Required quantities of glyceryl monostearate (GMS), light liquid paraffin, isopropyl palmitate, emulsifying wax, stearic acid, and cetyl alcohol were weighed and transferred into a 200 ml beaker. To this, a required amount of jojoba oil and Vitamin E were added and heated in a water bath. Another 200 ml beaker was weighed, and required quantities of glycerin, TEA, methylparaben, and propylparaben were added followed by *A. vera* extract, grape seed extract, and tender coconut water. The beaker was heated to a temperature up to 60–70°C. After both the solutions had attained their respective temperature, beaker was removed from heat and poured the oily phase to water phase with continuous stirring. The stirring was continued until the mixture forms a creamy emulsion. A few drops of perfume with a small quantity of propylene glycol were mixed and were added to the creamy emulsion after cooling (Table 1).

Formulation of gels

Antiaging gel was prepared using carbopol 940 as the gelling agent. Gelling agent was dispersed in a small quantity of distilled water and then stored overnight to ensure complete hydration. The active ingredients such as tender coconut water, *A. vera* extract, grape seed extract, Vitamin E, and jojoba oil in a suitable solvent such as propylene glycol were added to the dispersion. Other excipients such as methylparaben and propylparaben were also added slowly with continuous stirring. In carbopol gels, pH of the vehicle was brought to neutral by adding TEA. The final weight of the gel was adjusted to 50 g with distilled water. Entrapped air bubbles were removed by keeping the gels in vacuum desiccator (Table 2).

Formulation of phytosomes

Phytosomes are prepared by reacting the herbal extract in an aprotic solvent such as methylene chloride, dioxane, and ethyl acetate with the phospholipid such as phosphatidylcholine, phosphatidyl ethanolamine, or phosphatidylserine dissolved in the same solvent. After solubilization has completed, the complex compounds are isolated by removing the solvent under vacuum, by freeze drying or by precipitation with non-solvents such as n-hexane. Thus, the obtained complexes are lipophilic in character and soluble in a polar and aprotic solvent, in which the individual components of the complex are normally insoluble [62].

Formulation of phytosomal gel

Phytosomes are prepared by reacting the herbal extract and phospholipid such as soy lecithin in a ratio of 1:1 and dissolving them in an aprotic solvent such as ethyl acetate. After solubilization has completed, the complex compounds are removed by solvent evaporation technique. Thus, phytosomes are obtained. Gel was prepared using carbopol 940 as the gelling agent which was dispersed in a small quantity of distilled water and then stored overnight to ensure complete hydration. The active ingredients such as tender coconut water, *A. vera* extract, grape seed extract, Vitamin E, and jojoba oil in a suitable solvent such as propylene glycol were added to the dispersion. Then, preservatives such as methylparaben and propylparaben were also added slowly with continuous stirring. Then, the prepared phytosomes were incorporated into the gel, and thus, the phytosomal gel was obtained. This phytosomal gel showed better release of herbal extracts and better penetration to the skin, and as a result, desired antiaging property was obtained.

Evaluation studies

In vitro antioxidant studies [40]

2,2-diphenylpicrylhydrazyl (DPPH) free radical scavenging assay [41]
Different concentrations of standard ascorbic acid and sample, namely, 20, 40, 60, 80, and 100 mcg/ml were prepared in methanol. 0.002% DPPH in methanol was used as free radical. Equal volume of different concentrations of standards and DPPH was mixed in a clean and labeled test tubes separately, and the tubes were incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using UV-Vis spectrophotometer. The degree of stable DPPH* decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the sample. The scavenging activity of the sample against the stable DPPH* was calculated using the following equation:

Scavenging activity (%)=(A−B/A) 100

Where A is absorbance of control and B is absorbance of sample.

Reducing power assay
Different concentrations of standard ascorbic acid and sample, namely, 20, 40, 60, 80, and 100 mcg/ml in 1ml of methanol were mixed with 2.5ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide separately. The mixtures were placed in a water bath for 20 min at 50°C, cooled rapidly, mixed with 2.5 ml of 10% tri

Table 1: Composition of antiaging cream

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
GMS (g)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Isopropyl palmitate (ml)	1	1	1	1	1	1	1	1	1
Light liquid paraffin (ml)	1	1	1	1	1	1	1	1	1
Emulsifying wax (g)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Stearic acid (g)	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Cetyl alcohol (g)	1	1	1	1	1	1	1	1	1
Glycerin (ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Triethanolamine (ml)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methylparaben (g)	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Propylparaben (g)	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
TCW (ml)	40	40	40	40	40	40	40	40	-
Aloe vera (ml)	1.25	1.25	-	-	1.25	1.25	-	-	-
Grape seed extract (g)	0.013	-	0.013	-	0.013	-	0.013	-	-
Vitamin E (ml)	0.005	-	-	0.005	-	0.005	0.005	-	-
Jojoba oil (ml)	1	1	1	1	1	1	1	1	1
PG (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

GMS: Glyceryl monostearate

Table 2: Composition of antiaging gel

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Carbopol 934	1 g	1 g	1 g	1 g	1 g	1 g	1 g	1 g	1 g
Methyl paraben (0.5%)	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Propylparaben (0.2%)	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Propylene glycol 400 (5%)	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
Triethanolamine	1.2 ml	1.2 ml	1.2 ml	1.2 ml	1.2 ml	1.2 ml	1.2 ml	1.2 ml	1.2 ml
Tender coconut water	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	-
Aloe vera extract	1 ml	1 ml	-	-	1 ml	1 ml	-	-	-
Grape seed extract	0.2 g	-	0.2 g	-	0.2 g	-	0.2 g	-	-
Vitamin E	0.1 ml	-	-	0.1 ml	0.1 ml	0.1 ml	0.1 ml	-	-
Jojoba oil	0.05 ml	0.05 ml	0.05 ml	0.05 ml	0.05 ml	0.05 ml	0.05 ml	0.05 ml	0.05 ml

chloroacetic acid, and 0.5 ml of 0.1% ferric chloride. The intensity of iron (II)–ferricyanide complex was determined by measuring the formation of Perl's Prussian blue color at 700 nm after 10 min. The higher absorbance of the reaction mixture indicates increased reducing power. The scavenging activity of the sample was calculated using the following equation:

Scavenging activity (%)=(A−B/A) 100

Where A is absorbance of control and B is absorbance of sample.

Nitric oxide scavenging assay

Sodium nitroprusside (5 µM) in standard phosphate-buffered solution (PBS) was incubated with different concentration of the samples dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated for 25°C for 5 h. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulfanilamide in 2% phosphoric acid and 0.1% M naphthyl ethylenediamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in place of samples. The activity was compared with the standard ascorbic acid, which was used a standard antioxidant.

Hydrogen peroxide assay

A solution of hydrogen peroxide (20 mM) was prepared in PBS (pH 7.4). Various concentrations of 1 ml of the samples or standards in methanol were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against blank solution that contained extracts in PBS without hydrogen peroxide. IC50 value is the concentration of the sample required to scavenge 50% free radical. The above experiments were performed (in triplicate) and the percentage inhibition was calculated using the following formula:

%scavenged $[H_2O_2]=[(A_0-A_1)/A_0]\times100$,

Where A_0 was the absorbance of the standard (ascorbic acid) and A_1 was the absorbance of samples.

Physical evaluation of antiaging cream and gel

Preliminary evaluation was carried out as follows:

pH

The pH meter was calibrated using standard buffer solution such as pH 4 and 7. About 0.5 g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured.

Viscosity

Viscosity of the formulation was determined by Brookfield Viscometer at 100 rpm, using spindle no 7.

Homogeneity

The formulations were tested for the homogeneity by visual appearance

Spreadability

Two glass slides of 20 cm × 20 cm were selected. A small amount of sample was sandwiched between the two glass slides. A 100 g weight

was placed on the upper slide so that the cream between the two slides was pressed uniformly to form a thin layer. The weight was removed and then fixed to a stand without slightest disturbance in such a way that the upper slide slides off freely, to the force of weight tied to it. The time taken for the upper slide to separate away from the lower one was noted using a stop clock. This parallel plate method is the most widely used method for determining and quantifying the spreadability of semisolid preparations. Simplicity and relative lack of expense are the advantages of this method. The following equation was used for this purpose:

$S = m \times L/T$

Where

S - Spreadability

m - Weight tied to the upper slide l - Length of the glass

t - Time taken in seconds.

Extrudability

It is an empirical test to measure the force required for the cream to extrude out from the tube. The prepared cream was filled into a collapsible tube and it was sealed and the weight of the tube was recorded. Placed a 500 g weight on the tube and the amount of cream that extruded out was collected and weighed. Then, the percentage of cream extruded was calculated. The packing of creams has gained a considerable importance in the delivery of desired quantity of cream; therefore, measurement of extrudability has become some important criteria for creams.

Characterization of phytosomal gel

The behavior of phytosomes in both physical and biological system is governed by the factors such as physical size, shape, stability, and its distribution. Therefore, the phytosomes are characterized for physical attributes, i.e. shape, size, and its distribution.

Visualization

Visualization of phytosome can be achieved using scanning electron microscopy (SEM).

Vesicle size and zeta potential

The particle size and zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy.

Vesicle stabil*ity*

The stability of vesicles can be determined by assessing the size of the vesicles overtime. Mean size is measured by DLS.

Comparison studies of antiaging cream, gel, and phytosomal gel

The prepared formulations of cream, gel, and phytosomal gel were compared to find out which formulation had the highest antioxidant activity. The comparison study was done on the basis of *in vitro* antioxidant models.

Comparison studies based on the IC50 values of each formulation

A comparative study was also done on the basis of IC50 value of each formulation. The IC50 value is the concentration of the sample which scavenges 50% of the free radicals. The lower the IC50 value, the highest the antioxidant activity.

Stability studies

Stability studies were performed according to the ICH guidelines. The optimized formulation was kept at two different temperatures 30°C ± 2°C and 4°C ± 2°C for 45 days.

Comparison of the optimized phytosomal gel with marketed antiaging gel

The prepared antiaging phytosomal gel was compared with a marketed formulation, antiaging *A. vera* gel. This marketed formulation contained *A. vera* as antiaging ingredient because of the presence of phenol compounds and Vitamin E and C. The phytosomal gel containing tender coconut water and *A. vera* extract was compared with the antiaging *A. vera* gel.

RESULTS AND DISCUSSIONS

Physicochemical parametric evaluation studies

Physicochemical parameters such as pH, viscosity, homogeneity, spreadability, and extrudability of antiaging creams and gels were found out, and it was found that F2 formulation showed optimum value.

Physicochemical parameter evaluation of antiaging cream

pH measurement

It was previously reported that, for creams and gels to be non-irritant and safe for topical application, their pH has to be fall in the physiologic accepted range for topical preparations, i.e., pH 6–7 units. Table 3 shows that pH of various antiaging cream formulations ranged from 6.1 to 6.5 which lies in the normal physiologic range and thus produces no skin irritation.

Viscosity

The prepared antiaging creams and gels were formulated using Carbopol 940. Table 4 shows that the viscosity of various antiaging cream formulations ranged from 2455.577 to 5134.42.

Spreadability

The spreadability is an important criterion for uniform and ease of application of topical preparations. It also plays a major role from patient compliance point of view. Application of the formulation to the skin is more comfortable if the base spreads easily, exhibiting maximum "slip" and "drag." Spreadability of creams and gels are measured in terms of average diameter of the spread circle. Table 5 shows that

Table 3: pH of various antiaging cream formulations

Formulation code	pH			Average pH
	Trial 1	Trial 2	Trial 3	
F1	6.4	6.5	6.6	6.5±0.1
F2	6.2	6.4	6.6	6.4±0.2
F3	6	6.2	6.4	6.2±0.2
F4	6.1	6.3	6.4	6.2±0.15
F5	6	6.1	6.2	6.1±0.1
F6	6.4	6.5	6.6	6.5±0.1
F7	6.5	6.7	6.8	6.6±0.15
F8	6.2	6.3	6.4	6.3±0.1
F9	6.1	6.2	6.4	6.2±0.5

The values are expressed as mean±SD. SD: Standard deviation

Table 4: Viscosity of various antiaging cream formulations

Formulation code	Viscosity (cps)			Average viscosity (cps)
	Trial 1	Trial 2	Trial 3	
F1	2453.33	2462.88	2450.52	2455.577±6.4
F2	2454.21	2471.89	2454.64	2465.288±6.7
F3	2461.19	2482.91	2468.75	2481.387±6.8
F4	2494.74	2487.43	2499.13	2490.476±5.9
F5	2498.82	2489.51	2499.82	2491.534±4.3
F6	3289.76	3277.56	3284.10	3283.807±6.1
F7	3294.88	3279.67	3292.48	3287.743±5.2
F8	3414.72	3420.45	3417.04	3417.403±2.8
F9	5132.78	5129.78	5140.71	5134.423±5.6

The values are expressed as mean±SD. SD: Standard deviation

the spreadability values for all prepared cream formulations ranged 23.4–35.7

Extrudability

It is an empirical test to measure the force required for the cream to extrude out from the tube. For topical preparations, it is an important criterion to check the easiness of the cream to extrude out from the tube. Table 6 shows that the extrudability values of cream are ranged from 90.5 to 93.5.

Physicochemical parameter evaluation of antiaging gels

pH measurement

Table 7 shows that pH of various antiaging gel formulations ranged from 6.13 to 6.5 which lies in the normal physiologic range and thus produces no skin irritation.

Viscosity

Table 8 shows that the viscosity of various antiaging gel formulations ranged from 2446.577 to 5334.423.

Spreadability

Table 9 shows the spreadability values for all prepared gel formulations ranged from 22.4 to 38.2

Extrudability

Table 10 shows that the extrudability values of all prepared gel formulations lie from 90.5 to 93.5.

***In vitro* antioxidant studies**

In vitro antioxidant studies were performed for the prepared antiaging creams, gels, and phytosomal gels. The antioxidant activity of the prepared formulations in various concentrations was evaluated using *in vitro* models. It was observed that the test compounds scavenged free radicals in concentration-dependent manner in all models. The antioxidant activity was expressed as IC_{50} (the amount of antioxidant needed to decrease the radical concentration by 50%), which is negatively related to antioxidant activity. The lower the IC_{50} value, the higher is the antioxidant activity of the tested sample. The *in vitro* antioxidant studies included DPPH assay, reducing power assay, nitric oxide scavenging assay, and hydrogen peroxide assay. Among them, DPPH assay method was found to be the easiest, sensitive, and

Table 5: Spreadability of various antiaging cream formulations

Formulation code	Spreadability (g/s)			Average spreadability (g/s)
	Trial 1	Trial 2	Trial 3	
F1	32.2	32.4	32.6	32.4±0.2
F2	35.5	35.7	35.9	35.7±0.2
F3	31.1	31.2	31.3	31.2±0.1
F4	34.9	35	36.5	35.4±0.89
F5	26.2	27.3	28.7	27.4±1.25
F6	24.3	25.4	26.5	25.4±1.1
F7	28.4	29.1	30.8	29.4±1.2
F8	22.4	23.3	24.6	23.4±1.1
F9	29.9	30.2	31.8	30.6±1.02

The values are expressed as mean±SD. SD: Standard deviation

Table 6: Extrudability of various antiaging cream formulations

Formulation code	Extrudability			Average extrudability
	Trial 1	Trial 2	Trial 3	
F1	89.9	90.7	91.2	90.6±0.6
F2	89.5	90.9	91.3	90.5±0.9
F3	91.2	92.8	93.4	92.4±1.13
F4	91.2	92.9	93.8	92.6±1.32
F5	92.8	93.2	94.5	93.5±0.88
F6	92.4	93.1	94.5	93.3±1.06
F7	90.2	91.6	92.3	91.3±1.06
F8	92.3	93.9	94.5	93.5±1.13
F9	90.3	91.8	92.3	91.3±1.06

The values are expressed as mean±SD. SD: Standard deviation

Table 7: pH of various antiaging gel formulations

Formulation code	pH			Average pH
	Trial 1	Trial 2	Trial 3	
F1	6.4	6.5	6.6	6.5±0.1
F2	6.3	6.4	6.5	6.4±0.1
F3	6.1	6.3	6.5	6.3±0.2
F4	6	6.2	6.4	6.2±0.2
F5	6	6.1	6.3	6.13±0.15
F6	6.5	6.6	6.7	6.6±0.1
F70	6.5	6.7	6.9	6.7±0.2
F8	6.4	6.6	6.8	6.6±0.2
F9	6.1	6.2	6.3	6.2±0.1

The values are expressed as mean±SD. SD: Standard deviation

Table 8: Viscosity of various antiaging gel formulations

Formulation code	Viscosity (cps)			Average viscosity (cps)
	Trial 1	Trial 2	Trial 3	
F1	2453.33	2462.88	2450.52	2446.577±6.4
F2	2454.21	2471.89	2454.64	2472.288±6.7
F3	2461.19	2482.91	2468.75	2480.387±6.8
F4	2494.74	2487.43	2499.13	2484.476±5.9
F5	2498.82	2489.51	2499.82	2499.534±4.3
F6	3289.76	3277.56	3284.10	3082.807±6.1
F7	3294.88	3279.67	3292.48	3184.743±5.2
F8	3414.72	3420.45	3417.04	3218.403±2.8
F9	5132.78	5129.78	5140.71	5334.423±5.6

The values are expressed as mean±SD. SD: Standard deviation

Table 9: Spreadability of various antiaging gel formulations

Formulation code	Spreadability (g/sc)			Average spreadability (g/s)
	Trial 1	Trial 2	Trial 3	
F1	32.2	32.4	32.6	31.4±0.2
F2	35.5	35.7	35.9	33.7±0.2
F3	31.1	31.2	31.3	38.2±0.1
F4	34.9	35	36.5	34.4±0.89
F5	26.2	27.3	28.7	23.4±1.25
F6	24.3	25.4	26.5	22.4±1.1
F7	28.4	29.1	30.8	27.4±1.2
F8	22.4	23.3	24.6	25.4±1.1
F9	29.9	30.2	31.8	36.6±1.02

The values are expressed as mean±SD. SD: Standard deviation

Table 10: Extrudability of various antiaging gel formulations

Formulation code	Extrudability			Average
	Trial 1	Trial 2	Trial 3	
F1	89.9	90.7	91.2	90.7±0.6
F2	89.5	90.9	91.3	90.5±0.9
F3	91.2	92.8	93.4	92.6±1.13
F4	91.2	92.9	93.8	92.8±1.32
F5	92.8	93.2	94.5	92.5±0.88
F6	92.4	93.1	94.5	93.2±1.06
F7	90.2	91.6	92.3	91.1±1.06
F8	92.3	93.9	94.5	93.5±1.13
F9	90.3	91.8	92.3	90.3±1.06

The values are expressed as mean±SD. SD: Standard deviation

rapid method to assess the antioxidant activity of compounds. Nine different formulations of antiaging creams, gels, and phytosomal gels were prepared. Although the radical scavenging activity of the standard was found to be higher than that of the samples, the study showed that the samples have the proton-donating ability and could serve as free radical inhibitors or scavengers. Among them, F2 formulation showed the highest antioxidant activity. Physicochemical parameters such as pH, viscosity, homogeneity, spreadability, and extrudability were evaluated for both antiaging creams and gels. From the physicochemical parameter evaluation, it was found that F2 sample showed optimum values. Hence, the F2 formulation was taken as the optimized formula from all the nine different formulations. Characterization studies of antiaging phytosomal gel were also performed.

Antiaging cream

DPPH free radical scavenging activity

The result of antioxidant activity of different concentrations of samples and standard (ascorbic acid) is shown in Table 11. The samples exhibited marked antioxidant activity by scavenging DPPH* (free radical) and converting into DPPHH. A dose-dependent radical scavenging activity was observed. The scavenging activity of ascorbic acid was greater than that of all the nine different samples of cream. Among them, F2 sample showed the highest antioxidant activity. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases.

In this study, the scavenging activity of F2 sample was found to be dose dependent, i.e., higher the concentration, more was the scavenging activity. Although the DPPH radical scavenging abilities of the samples were less than that of ascorbic acid, the study showed that the samples have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Reducing power assay

The result of antioxidant activity of different concentrations of samples and standard (ascorbic acid) is shown in Table 12. In this study, the absorbance was found to increase with the dose of samples and standard which is suggestive of reducing power. In the Fe+3 reducing assay, the reducing power of antiaging cream and gel samples was found to increase with the dose. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe3+/ferricyanide complex to the ferrous form. Therefore, Fe2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the antioxidant activity of putative antioxidants has been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical.

Fig. 2 shows the reducing power assay of antiaging cream when compared with that of the standard ascorbic acid.

Nitric oxide scavenging assay

The result of antioxidant activity of different concentrations of samples and standard (ascorbic acid) is shown in Table 13. Nitric oxide free

Table 11: DPPH scavenging assay of antiaging cream and the standard ascorbic acid

Concentration	Standard	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
20	26.54±1.38	20.1±1.30	24.08±1.41	22.04±1.90	21.98±1.32	20.77±1.37	20.62±1.48	20.33±1.48	20.21±1.49	20.19±1.46
40	38.87±1.38	30.06±1.42	36.68±1.41	33.99±1.32	32.06±1.51	31.05±1.37	34.87±1.34	33.87±1.40	32.23±1.39	31.17±1.49
60	46.76±1.40	35.13±1.40	44.45±1.42	41.06±1.49	40.56±1.32	40.77±1.35	42.02±1.32	42.28±1.36	42.19±1.38	40.01±1.49
80	56.66±1.39	43.9±1.36	55.01±1.47	53.67±1.30	52.34±1.29	51.09±1.31	53.66±1.36	53.82±1.22	53.34±1.29	50.87±1.39
100	58.87±1.41	51.03±1.45	56.98±1.41	52.99±1.37	51.09±1.39	50.06±1.39	52.09±1.37	52.11±1.44	52.77±1.39	52.46±1.34

DPPH: 2,2-diphenylpicrylhydrazyl

Table 12: Reducing power assay of antiaging cream and the standard ascorbic acid

Concentration	Standard	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
20	25.88±1.49	22.98±±1.48	24.64±1.43	23.53±1.48	22.74±1.49	22.63±1.43	22.31±1.40	21.74±1 0.49	20.79±1.44	20.32±1.33
40	38.98±1.50	33.27±1.42	36.73±1.43	35.55±1.47	34.72±1.47	33.33±1.48	32.87±1.47	31 0.82±1 0.49	30.73±1.42	30.28±1.32
60	45.53±1.41	42.01±1.47	43.99±1.44	42.82±1.47	41.66±1.41	40.83±1.42	40.65±1.40	40 0.37±1 0.47	40.27±1.41	40.19±1.31
80	52.22±1.46	50.09±1.48	51.87±1.41	50.71±1.41	50.7±1.41	50.65±1.40	50.36±1.39	50 0.22±1 0.46	50.18±1.40	50.12±1.30
100	57.73±1.45	52.22±1.44	55.98±1.40	54.73±1.39	53.87±1.39	52.55±1.44	51.64±1.39	51 0.81±1 0.46	50.91±1.39	50.26±1.34

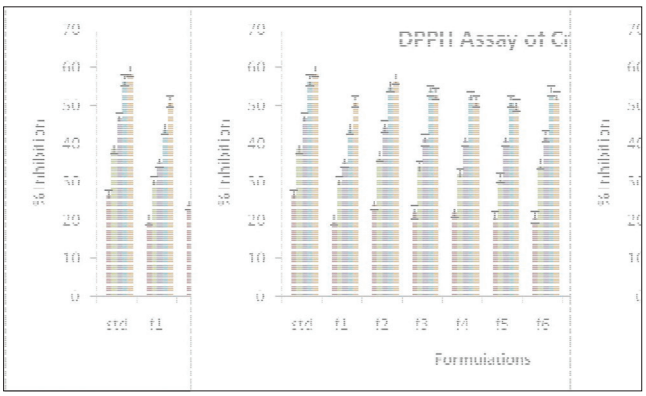

Fig. 1: Percentage inhibition of antiaging cream by 2,2-diphenylpicrylhydrazyl assay when compared with the standard

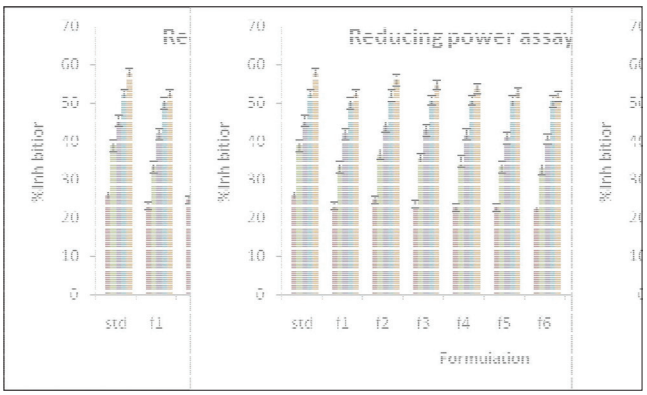

Fig. 2: Percentage inhibition of antiaging cream by reducing power assay when compared with the standard

radicals are released from the endothelial cells which are necessary for the inflammatory actions of skin. The results signify that the level of nitric oxide was reduced to a considerable amount by the samples. Thus, the prepared formulation can be used effectively for topical purposes.

Table 13 suggests the potency of nitric oxide scavenging activity of the formulations with that of the standard ascorbic acid.

Fig. 3 shows the nitric oxide scavenging assay of antiaging cream when compared with the standard.

Hydrogen peroxide assay

H_2O_2 is a weak oxidizing agent and can activate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen per oxide can cross cell membrane rapidly. Once inside the cell, H_2O_2 can probably react with $Fe2^+$ and possibly $Cu2^+$ to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The decomposition of hydrogen peroxide by the formulations may result from its antioxidant and free radical scavenging activity.

The results given in Table 14 show the percentage of hydrogen peroxide radical scavenged by the samples and ascorbic acid at increasing concentrations. From them, it is evident that the formulations were capable of scavenging hydrogen peroxide in a concentration-dependent manner (Fig. 4).

Antiaging Gel

DPPH assay of antiaging gels

Table 15 shows the percentage of DPPH free radicals scavenged by the sample and standard at increasing concentrations (Fig. 5).

Reducing power assay of gels

Table 16 shows the percentage of free radicals scavenged by the sample and standard at increasing concentrations (Fig. 6).

Nitric oxide scavenging assay of gels

Table 17 shows the percentage of free radicals scavenged by the sample and standard at increasing concentrations (Fig. 7).

Hydrogen peroxide assay of gels

Table 18 shows the percentage of free radicals scavenged by the sample and standard at increasing concentrations (Fig. 8).

Antiaging phytosomal Gel

DPPH assay of phytosomal gel

Table 19 shows the percentage of free radicals scavenged by the sample and standard at increasing concentrations (Fig. 9).

Reducing power assay of phytosomal gel

Table 20 shows the percentage of free radicals scavenged by the sample and standard at increasing concentrations (Fig. 10).

Table 13: Nitric oxide scavenging assay of antiaging cream and the standard ascorbic acid

Concentration	Standard	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
20	29.76±1.45	22.22±1.44	27.98±1.50	25.55±1.17	23.22±1.49	22.91±1.45	21.88±1.43	21.22±1.47	21.18±1.47	20.72±1.47
40	37.77±1.39	32.28±1.34	35.±1.42	33.97±1.44	32.01±1.48	31.19±1.44	30.63±1.48	30.55±1.41	30.43±1.41	30.36±1.47
60	49.37±1.42	42.71±1.45	47.24±1.43	45.13±1.44	43.87±1.49	42.66±1.41	41.72±1.46	40.73±1.38	40.54±1.38	40.27±1.47
80	57.92±1.43	53.76±1.37	55.46±1.48	54.37±1.45	53.82±1.46	52.74±1.43	51.28±1.48	50.82±1.43	50.55±1.49	50.21±1.45
100	60.98±1.50	55.55±1.47	57.77±1.45	54.82±1.46	52.11±1.50	51.82±1.40	50.73±1.47	50.65±1.41	50.53±1.41	50.19±1.43

Table 14: Hydrogen peroxide scavenging of antiaging cream and the standard ascorbic acid

Concentration	Standard	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
20	29.99±1.45	25.55±1.44	27.77±1.29	26.65±1.30	25.29±1.42	24.73±1.38	23.81±1.44	22.98±1.44	21.83±1.43	20.08±1.45
40	38.82±1.43	35.12±1.44	36.98±1.31	35.81±1.50	34.61±1.41	33.83±1.39	32.74±1.44	31.83±1.44	30.98±1.32	30.56±1.43
60	45.65±1.41	42.29±1.42	43.83±1.49	42.26±1.30	41.98±1.37	40.83±1.32	40.84±1.44	40.75±1.37	40.65±1.23	40.44±1.33
80	55.91±1.42	52.22±1.41	53.39±1.37	52.38±1.33	51.73±1.38	50.74±1.32	50.55±1.37	50.43±1.32	50.33±1.33	50.21±1.25
100	58.89±1.40	54.44±1.40	56.77±1.37	55.47±1.26	54.73±1.39	53.82±1.31	52.89±1.33	51.73±1.44	50.54±1.29	50.32±1.44

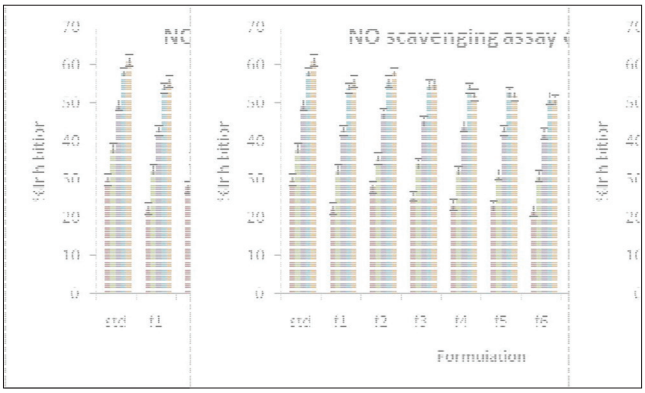

Fig. 3: Percentage inhibition of the formulation by nitric oxide scavenging assay when compared with the standard

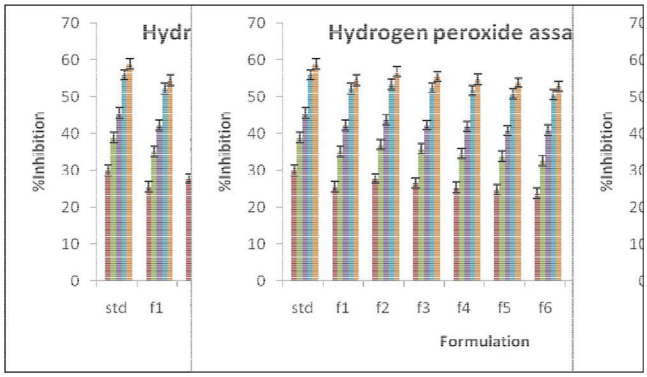

Fig. 4: Percentage inhibition of the formulation by hydrogen peroxide assay when compared with the standard

Table 15: DPPH assay of antiaging gel and standard ascorbic acid

Concentration	Standard	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
20	31.11 ±1.50	28.74 ±1.45	29.99 ±1.49	27.66 ±1.43	26.64 ±1.21	25.48 ±1.46	24.77 ±1.49	23.54 ±1.49	22.73 ±1.39	21.81 ±1.49
40	40.98 ±1.41	37.75 ±1.43	38.63 ±1.43	36.84 ±1.49	35.74 ±1.37	34.49 ±1.47	33.65 ±1.44	32.46 ±1.49	31.87 ±1.48	30.84 ±1.46
60	49.99 ±1.35	47.53 ±1.48	48.88 ±1.43	46.65 ±1.41	45.73 ±1.38	44.73 ±1.42	43.52 ±1.42	42.64 ±1.42	41.43 ±1.48	40.65 ±1.36
80	59.75 ±1.33	57.53 ±1.28	58.83 ±1.48	56.73 ±1.48	55.82 ±1.38	54.72 ±1.37	53.87 ±1.29	52.75 ±1.43	51.83 ±1.28	50.74 ±1.23
100	65.71 ±1.39	62.22 ±1.39	63.81 ±1.37	61.82 ±1.30	61.55 ±1.45	61.32 ±1.48	61.11 ±1.49	60.75 ±1.41	60.66 ±1.39	60.26 ±1.43

DPPH: 2,2-diphenylpicrylhydrazyl

Table 16: Reducing power assay of antiaging gel and standard ascorbic acid

Concentration	Standard	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
20	40.98±1.34	33.82±1.45	38.87±1.45	31.33±1.48	30.29±1.11	34.66±1.47	32.97±1.47	31.64±1.13	31.33±1.49	30.29±1.41
40	48.85±1.48	44.98±1.39	47.67±1.37	40.43±1.34	40.28±1.05	45.57±1.48	43.74±1.40	41.85±1.17	40.43±1.41	40.28±1.47
60	59.83±1.32	55.83±1.32	58.64±1.34	51.82±1.28	50.47±1.20	56.83±1.28	54.82±1.41	52.64±1.13	51.82±1.44	50.47±1.38
80	68.83±1.40	64.85±1.41	67.75±1.36	60.38±1.34	60.11±1.14	65.74±1.32	63.71±1.28	6 0.72±1.17	60.38±1.27	60.11±1.32
100	73.39±1.42	70.22±1.47	72.29±1.28	70.05±1.43	70.18±1.01	70.11±1.47	70.21±1.34	70.09±1.13	70.05±1.39	70.18±1.41

Table 17: Nitric oxide scavenging assay of antiaging gel and standard ascorbic acid

Concentration	Standard	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
20	40.98±1.23	33.82±1.39	37.98±1.34	34.72±1.39	34.66±1.29	32.97±1.18	31.83±1.44	31.64±1.33	31.33±1.46	30.29±1.32
40	48.85±1.44	44.98±1.34	46.87±1.41	45.65±1.28	45.57±1.41	43.74±1.38	42.75±1.38	41.85±1.48	40.43±1.32	40.28±1.33
60	59.83±1.47	55.83±1.34	57.64±1.38	56.68±1.27	56.83±1.34	54.82±1.34	53.73±1.37	52.64±1.30	51.82±1.30	50.47±1.24
80	68.83±1.40	64.85±1.36	66.66±1.31	65.87±1.31	65.74±1.32	63.71±1.32	62.61±1.26	61.72±1.30	60.38±1.33	60.11±1.43
100	73.39±1.41	70.22±1.38	72.77±1.38	70.36±1.30	70.11±1.38	70.21±1.39	70.11±1.29	70.09±1.26	70.05±1.31	70.18±1.47

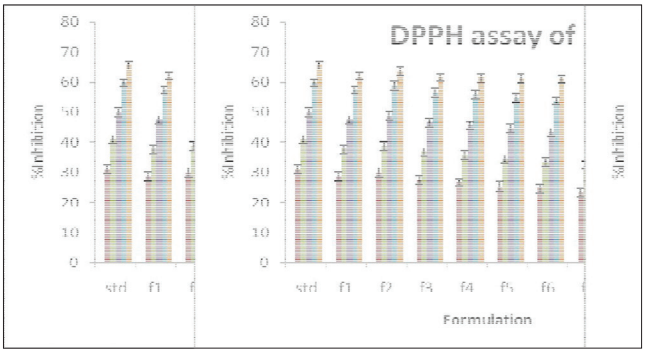

Fig. 5: Percentage inhibition of the formulation by2,2-diphenylpicrylhydrazyl assay when compared with the standard

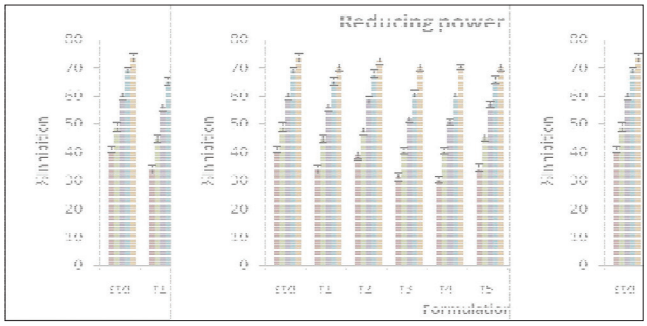

Fig. 6: Percentage inhibition of the formulation by reducing power assay when compared with the standard

Nitric oxide assay of phytosomal gels

Table 21 shows the percentage of free radicals scavenged by the sample and standard at increasing concentrations.

Hydrogen peroxide assay of phytosomal gel

Table 22 shows the percentage of free radicals scavenged by the sample and standard at increasing concentrations (Fig. 11).

All the formulations showed potent antioxidant activity in all the assay methods. From the figures, the result represents that all the nine formulations had the capacity to scavenge the free radicals, but among them, the F2 formulation containing both tender coconut water and *A. vera* extract showed the highest antioxidant activity and also the radical scavenging activity by DPPH assay method showed highest antioxidant activity than the other methods. Hence, it was concluded that the prepared phytosomal gel could be used for the purpose of anti-wrinkle treatment as it could scavenge the harmful free radicals.

IC_{50} values of optimized formulation, F2 of antiaging cream, gel, and phytosomal gel

IC_{50} value of antiaging cream, F2

IC_{50} value, i.e. the concentration of the sample which is required to scavenge 50% of the free radicals of the optimized formulation of the prepared antiaging cream, F2 is found to be 70.5 µg/ml (Fig. 12).

Table 18: Hydrogen peroxide assay of antiaging gel and standard ascorbic acid

Concentration	Standard	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
20	40.98±1.14	34.66±1.37	39.99±1.50	33.82±1.49	32.97±1.46	31.33±1.44	30.09±1.45	31.64±1.44	31.33±1.44	30.29±1.13
40	48.85±1.40	45.57±1.37	47.73±1.33	44.98±1.36	43.74±1.41	40.43±1.41	40.32±1.39	41.85±1.34	40.43±1.42	40.28±1.13
60	59.83±1.45	56.83±1.41	55.38±1.38	55.83±1.43	54.82±1.38	51.82±1.30	50.18±1.43	52.64±1.36	51.82±1.45	50.47±1.12
80	68.83±1.39	65.74±1.38	64.22±1.38	64.85±1.39	63.71±1.46	60.38±1.39	60.19±1.40	61.72±1.45	60.38±1.34	60.11±1.19
100	73.39±1.38	70.11±1.38	70.01±1.39	70.22±1.37	70.21±1.33	70.05±1.37	70.01±1.41	70.09±1.37	70.05±1.38	70.18±1.12

Table 19: DPPH assay of phytosomal gel and standard ascorbic acid

Concentration	Standard	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
20	40.98±1.51	34.66±1.44	35.98±1.50	34.72±1.45	33.82±1.49	32.97±1.42	31.83±1.50	31.64±1.40	31.33±1.34	30.29±1.49
40	48.85±1.45	45.57±1.39	46.87±1.39	45.65±1.33	44.98±1.36	43.74±1.42	42.75±1.37	41.85±1.39	40.43±1.39	40.28±1.40
60	59.83±1.35	56.83±1.44	57.99±1.40	56.68±1.34	55.83±1.39	54.82±1.33	53.73±1.35	52.64±1.28	51.82±1.39	50.47±1.39
80	68.83±1.38	65.74±1.41	66.72±1.28	65.87±1.36	64.85±1.38	63.71±1.40	62.61±1.39	61.72±1.44	60.38±1.47	60.11±11.40
100	73.39±1.34	70.81±1.37	71.21±1.35	70.36±1.45	70.22±1.30	70.21±1.39	70.11±1.45	70.09±1.47	70.05±1.31	70.18±1.38

DPPH: 2,2-diphenylpicrylhydrazyl

Table 20: Reducing power assay of phytosomal gel and standard ascorbic acid

Concentration	Standard	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
20	40.98±1.23	33.82±1.39	37.98±1.34	34.72±1.39	34.66±1.29	32.97±1.18	31.83±1.44	31.64±1.33	31.33±1.46	30.29±1.32
40	48.85±1.44	44.98±1.34	46.87±1.41	45.65±1.28	45.57±1.41	43.74±1.38	42.75±1.38	41.85±1.48	40.43±1.32	40.28±1.33
60	59.83±1.47	55.83±1.34	57.64±1.38	56.68±1.27	56.83±1.34	54.82±1.34	53.73±1.37	52.64±1.30	51.82±1.31	50.47±1.24
80	68.83±1.40	64.85±1.36	66.66±1.31	65.87±1.31	65.74±1.32	63.71±1.32	62.61±1.26	61.72±1.30	60.38±1.33	60.11±1.43
100	73.39±1.41	70.22±1.38	72.77±1.38	70.36±1.30	70.11±1.38	70.21±1.39	70.11±1.29	70.09±1.20	70.05±1.30	70.18±1.47

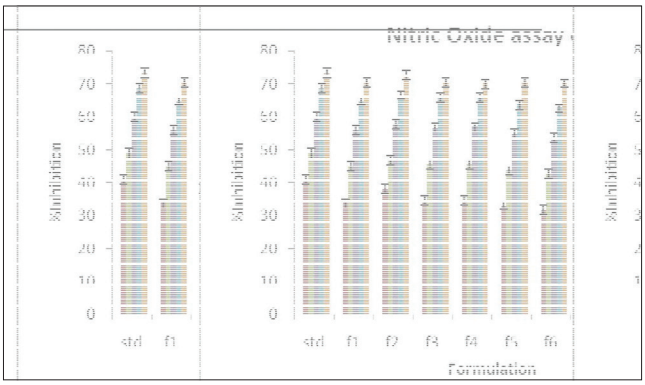

Fig. 7: Percentage inhibition of the formulation by nitric oxide scavenging assay when compared with the standard

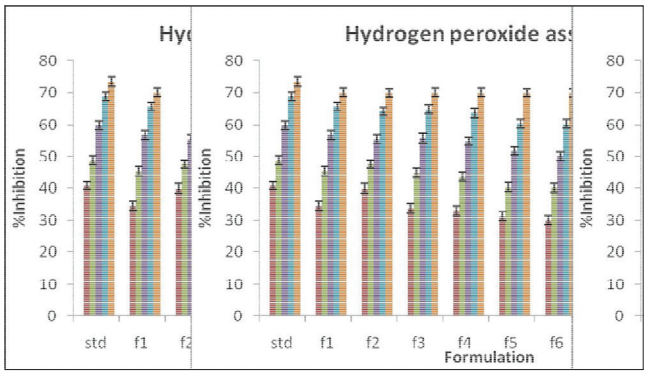

Fig. 8: Percentage inhibition of the formulation by hydrogen peroxide assay when compared with the standard

IC_{50} value of antiaging Gel, F2

IC_{50} value, i.e., concentration of the sample which is required to scavenge 50% of the free radicals of the optimized formulation of the prepared antiaging gel, F2 is found to be 65.05 µg/ml (Fig. 13).

IC_{50} value of antiaging phytosomal gel, F2

IC_{50} value, i.e., concentration of the sample which is required to scavenge 50% of the free radicals of the optimized formulation of the prepared antiaging phytosomal gel, F2 is found to be 47.0 µg/ml (Fig. 14).

Comparison studies of antiaging cream, gel, and phytosomal gel on the basis of in vitro antioxidant studies

The antioxidant property of the prepared formulations of antiaging cream, gel, and phytosomal gel was compared on the basis of *in vitro* antioxidant studies. From the comparison study, it was clear that antiaging. Phytosomal gel had the highest antioxidant activity, and thus, it could be concluded that the prepared phytosomal gel possessed the antiaging property. Comparison studies are shown in Table 23.

Fig. 15 indicates the graph in which it is clear cut that the antiaging phytosomal gel had the highest antioxidant activity.

Comparison studies of IC_{50} values of antiaging cream, gel, and phytosomal gel [41]

IC_{50} values of the optimized formulations (F2) of antiaging cream, gel, and phytosomal gel were compared. The IC_{50} value of antiaging cream was found to be 70.5 µg/ml, the IC_{50} value of antiaging gel was found to be 65.0 µg/ml, and the IC_{50} value of antiaging phytosomal gel was found to be 47.0 µg/ml. Therefore, it can be concluded that the antiaging phytosomal gel proved to show the highest antioxidant activity as it had an IC_{50} value of 47.0 µg/ml. The lower the IC_{50} value, the higher the antioxidant activity (Fig. 16).

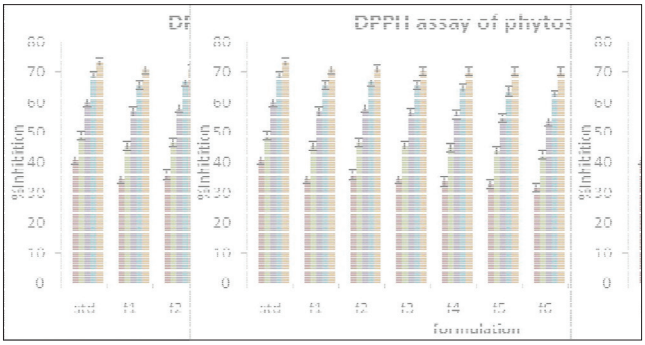

Fig. 9: Percentage inhibition of the formulation by 2,2-diphenylpicrylhydrazyl assay when compared with the standard

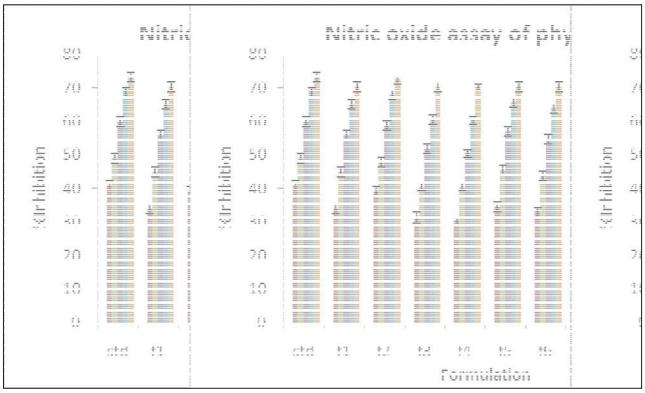

Fig. 10: Percentage inhibition of the formulation by nitric oxide scavenging assay when compared with the standard

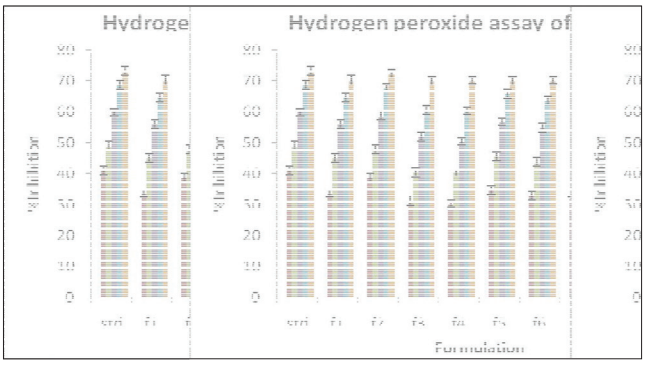

Fig. 11: Percentage inhibition of the formulation by hydrogen peroxide assay when compared with the standard

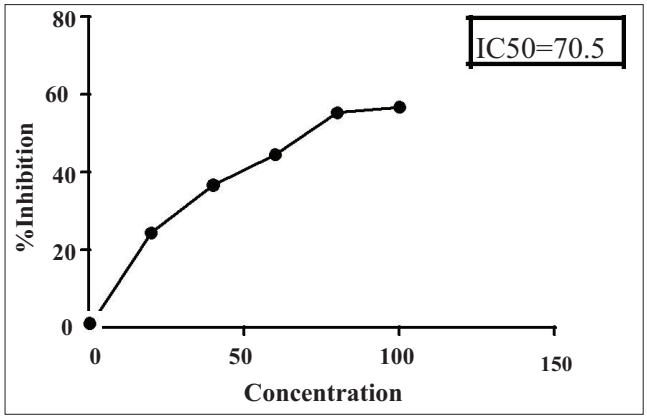

Fig. 12: The IC_{50} value of antiaging cream

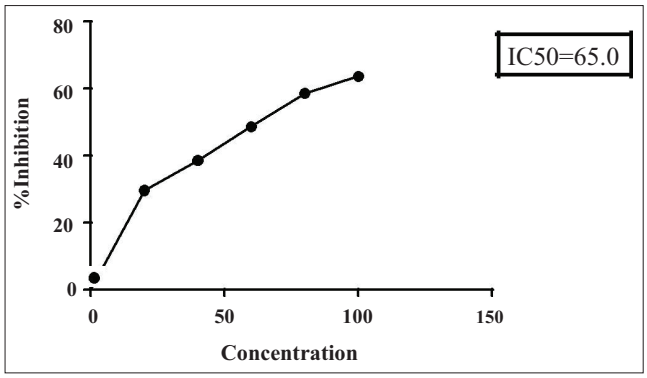

Fig. 13: The IC_{50} value of antiaging gel

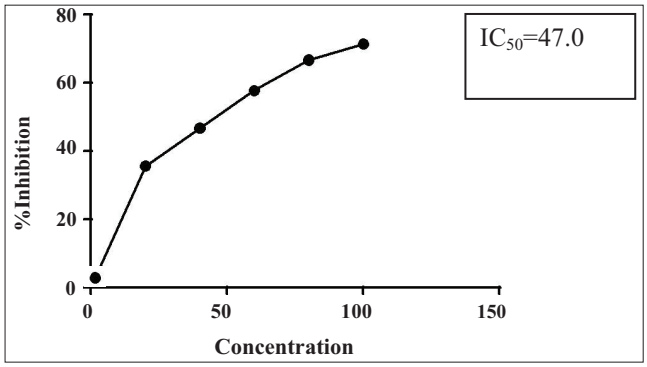

Fig. 14: The IC_{50} value of antiaging phytosomal gel

Characterization of antiaging phytosomal gel

Visual inspection

The prepared phytosomal gel was visually inspected and it showed that the formulation was homogeneous without any gritty particles and was of optimum consistency.

Globule size determination

Microscopic evaluation

The prepared antiaging phytosomal gel was observed under optical microscope at 100× and observed that the formed vesicles were of uniform size.

Size and size distribution:

The vesicular size and size distribution was evaluated using DLS, and the results showed that increase in extract phospholipid concentration increases the mean vesicular size. The vesicular size was between 52 nm and 115 nm. The polydispersity index was found to be low, shows that the particles were of low value and phytosomes formed by hydration was of uniform size (Figs. 17-21 and Table 24).

Zeta potential determination

The magnitude of zeta potential gives a potential stability of the colloidal dispersion. If the particles have large positive or negative charge reveals that they repel each other and there is dispersion stability. The zeta potential of the optimized formulation showed that the sample is sample which is highly stable. It was found as −22.21, and hence, this indicates that the prepared formulation is stable.

SEM

From Fig. 22, it is clear that the particle size of the optimized formulation was confirmed to be 52–115 nm. This was in accordance with the particle size of phytosomes in the literature.

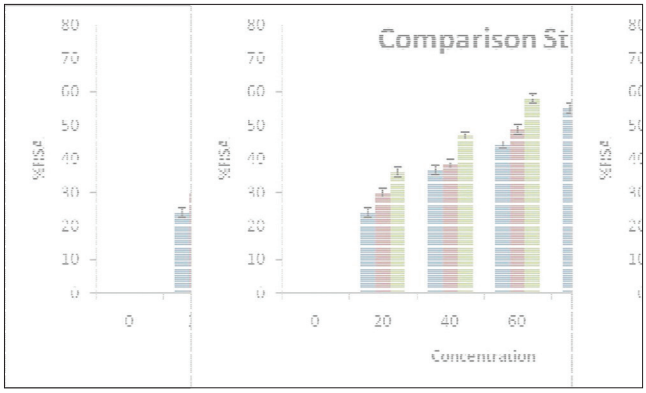

Fig. 15: Comparison studies of antiaging cream, gel, and phytosomal gel

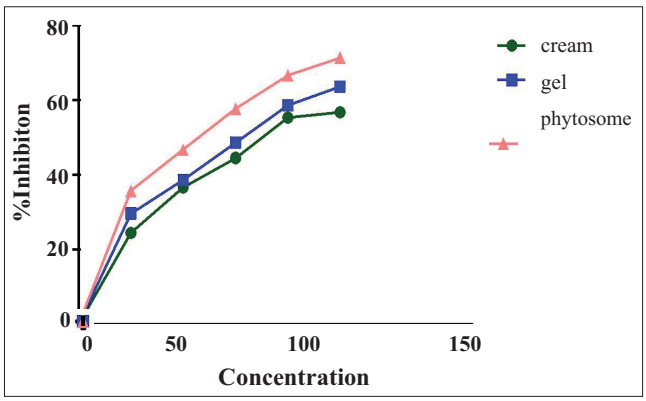

Fig. 16: The comparison studies of IC $_{50}$ values of antiaging cream, gel, and phytosomal gel

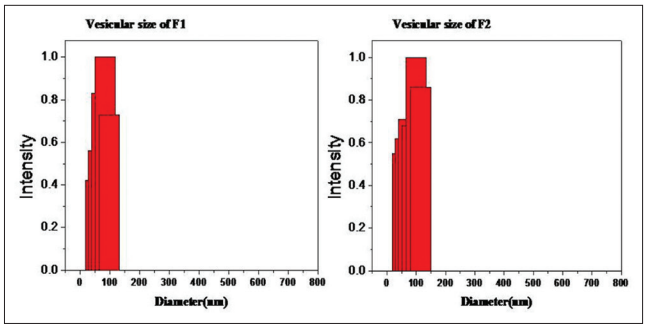

Fig. 17: Vesicular size of phytosomes F1-F2

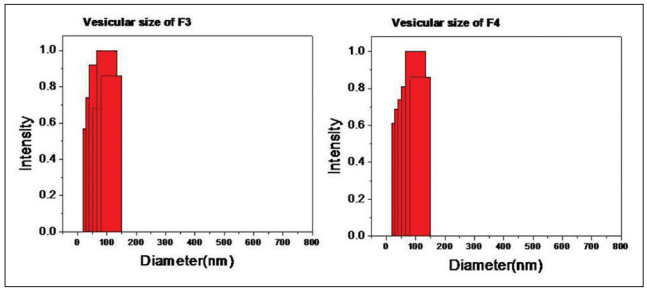

Fig. 18: Vesicular size of phytosomes F3-F4

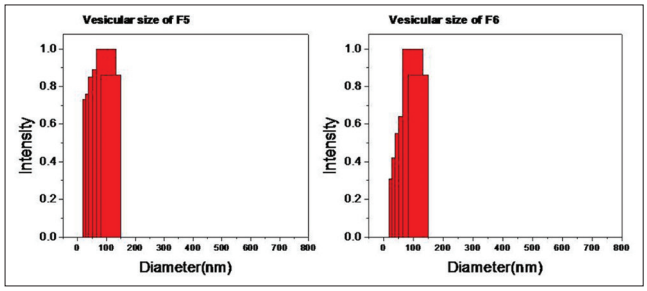

Fig. 19: Vesicular Size of Phytosome F5-F6

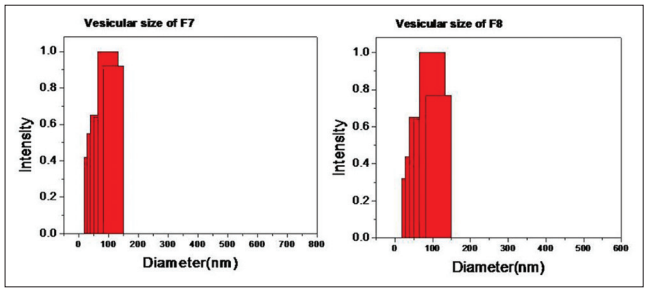

Fig. 20: Vesicular Size of Phytosome F7-F8

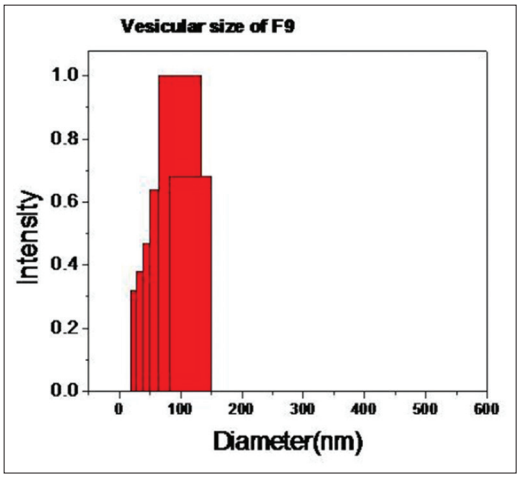

Fig. 21: Vesicular Size of Phytosome F9

Stability studies

The best formulation of the phytosomal gel was kept at varying conditions of temperature. The system was stable at 25°C. There were no significant changes in the formulation when kept at room temperature (30°C±2°C) and also at refrigerated temperature (4°C±2°C). No much change of pH, viscosity, homogeneity, spreadability, extrudability, and degradation of the samples were observed during 45 days period. Table 25 shows the data for stability studies for antiaging phytosomal gel F2. There was no much change in the zeta potential of the sample, and this proves that the phytosomal gel system remains stable.

Comparison of the phytosomal gel with marketed formulation

The prepared antiaging phytosomal gel was compared with that of an herbal marketed formulation, antiaging *A. vera* gel. It was found that the antioxidant activity was more for the phytosomal gel. Hence, it was proved that the prepared phytosomal gel containing the antiaging ingredients such as tender coconut water and *A. vera* had significant antiaging properties. The *A. vera* antiaging gel was taken for comparison as it had the antiaging ingredient, *A. vera*.

From Fig. 23, it was evident that the prepared antiaging phytosomal gel had the highest antioxidant property when compared with the conventional dosage forms such as antiaging cream and antiaging gel.

Table 21: Nitric oxide scavenging assay of phytosomal gel and standard ascorbic acid

Concentration	Standard	F1	F2	F3	F4	F5	F6	F 7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
20	40.98±1.34	33.82±1.45	38.87±1.45	31.33±1.48	30.29±1.11	34.66±1.47	32.97±1.47	31.64±1.13	31.33±1.49	30.29±1.41
40	48.85±1.48	44.98±1.39	47.67±1.37	40.43±1.34	40.28±1.05	45.57±1.48	43.74±1.40	41.85±1.17	40.43±1.41	40.28±1.47
60	59.83±1.32	55.83±1.32	58.64±1.34	51.82±1.28	50.47±1.20	56.83±1.28	54.82±1.41	52.64±1.13	51.82±1.44	50.47±1.38
80	68.83±1.40	64.85±1.41	67.75±1.36	60.38±1.34	60.11±1.14	65.74±1.32	63.71±1.28	61.72±1.17	60.38±1.27	60.11±1.32
100	73.39±1.42	70.22±1.47	72.29±1.28	70.05±1.43	70.18±1.01	70.11±1.47	70.21±1.34	70.09±1.13	70.05±1.39	70.18±1.41

Table 22: Hydrogen peroxide assay of phytosomal gel and standard ascorbic acid

Concentration	Standard	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
20	40.98±1.34	33.82±1.45	38.87±1.45	31.33±1.48	30.29±1.11	34.66±1.47	32.97±1.47	31.64±1.13	31.33±1.49	30.29±1.41
40	48.85±1.48	44.98±1.39	47.67±1.37	40.43±1.34	40.28±1.05	45.57±1.48	43.74±1.40	41.85±1.17	40.43±1.41	40.28±1.47
60	59.83±1.32	55.83±1.32	58.64±1.34	51.82±1.28	50.47±1.20	56.83±1.28	54.82±1.41	52.64±1.13	51.82±1.44	50.47±1.38
80	68.83±1.40	64.85±1.41	67.75±1.36	60.38±1.34	60.11±1.14	65.74±1.36	63.71±1.28	61.72±1.17	60.38±1.27	60.11±1.32
100	73.39±1.42	70.22±1.47	72.29±1.28	70.05±1.43	70.18±1.01	70.11±1.47	70.21±1.34	70.09±1.13	70.05±1.39	70.18±1.41

Table 23: Comparison studies of antiaging cream, gel, and phytosomal gel

Concentration (mcg/ml)	Cream	Gel	Phytosomal gel
0	0	0	0
20	24.08	29.99	35.98
40	36.68	38.63	46.87
60	44.45	48.88	57.99
80	55.01	58.83	66.72
100	56.98	63.81	71.21

Table 24: Size distribution and polydispersity of formulations

S. No	Formulation code	Vesicular size (nm)*	Polydispersity**
1	F1	52.44±31.45	145
2	F2	53.23±36.79	1453
3	F3	98.28±48.36	1621
4	F4	65.45±25.46	08335
5	F5	76.56±46.79	1478
6	F6	107.89±36.57	1759
7	F7	103.28±21.45	1109
8	F8	115.14±26.79	1982
9	F9	110.23±21.34	1050

*Data obtained from Nicomp 380 DLS, **PI: Standard deviation/mean vesicular size

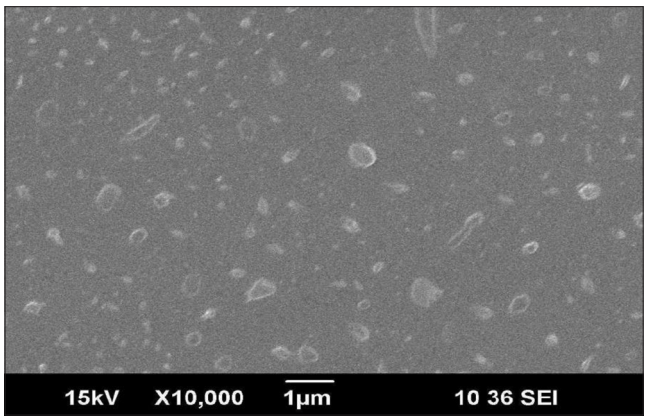

Fig. 22: Scanning electron microscopy of optimized formulation

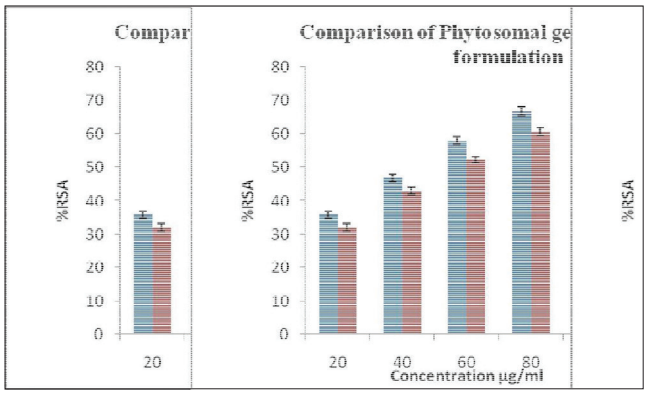

Fig. 23: The comparison between phytosomal gel and marketed formulation

CONCLUSION

Nowadays, aging phenomenon has become a severe problem among people. Hence, they started to depend on antiaging cosmetics. However, they had severe adverse effects, and hence, they had to rely on cosmetics containing herbal ingredients as they had fewer side effects. Antioxidants are the major ingredients present in antiaging cosmetics. The antioxidants chosen for this study were tender coconut water, *A. vera* extract, grape seed extract, and Vitamin E. Nine different formulations of antiaging cream, gel, and phytosomal gel were prepared. Physicochemical parameters such as pH, viscosity, homogeneity, spreadability, and extrudability of prepared antiaging cream and gel were determined and the formula, F2 containing both tender coconut water and *A. vera* extract was chosen as the optimized formula. *In vitro* antioxidant studies were performed for antiaging cream, gel, and phytosomal gel, and the phytosomal gel was found to be the formulation having the highest antioxidant activity, and hence, it was the optimized formulation. Characterization studies of the optimized formulation such as vesicle size, size distribution, and stability were performed, and it was identified that the prepared antiaging phytosomal gel had all the parameters as optimum. Stability studies were done according to the ICH guidelines, and it was found that the optimized formulation was stable under both room temperature and refrigerated temperature. The optimized formulation was compared with a marketed antiaging gel and it was clear cut that the prepared antiaging phytosomal gel had highest antioxidant activity than the marketed formulation, and hence, it could be used for antiaging treatment. The result revealed that the natural ingredients such as tender coconut water and *A. vera* extract had significant antioxidant activity and they were found to be potent scavengers of free radicals which serve as a possible preventative intervention for the skin diseases. The activity may be due to the presence of cytokinins, phenol compounds, and Vitamin E and C.

Table 25: Stability studies of phytosomal gel, F2

Days	Temperature	Formulation	Parameters				
			pH	Viscosity	Homogeneity	Spreadability	Extrudability
0	RT	1	6.5±0.1	23108±	Homogeneous	32.4±0.2	90.7±0.6
	30°C±2°C	2	6.4±0.2	27634±	Homogeneous	35.7±0.2	90.9±0.9
		5	6.2±0.1	30192±	Homogeneous	31.2±0.1	92.8±1.1
		8	6.3±0.1	35628±	Homogeneous	35±0.8	92.9±1.3
		1	6.1±0.1	26345±	Homogeneous	27.3±1.2	93.2±0.8
	4°C±2°C	2	6.5±0.1	26889±	Homogeneous	25.4±1.1	93.1±1.0
		5	6.7±0.1	27512±	Homogeneous	29.1±1.2	91.6±1.0
		8	6.8±0.1	28129±	Homogeneous	23.3±1.1	93.9±1.1
10	RT	1	6.7±0.1	30198±	Homogeneous	30.2±1.0	91.8±1.1
	30°C±2°C	2	6.5±0.1	31279±	Homogeneous	36.1±1.1	90.3±1.2
		5	6.2±0.2	35583±	Homogeneous	33.3±1.3	93.1±1.0
		8	6.3±0.2	39153±	Homogeneous	38.9±1.1	92.9±1.0
		1	6.8±0.2	27129±	Homogeneous	26.9±1.1	92.8±0.9
	4°C±2°C	2	6.3±0.1	27736±	Homogeneous	24.3±1.1	92.5±1.0
		5	6.1±0.1	28102±	Homogeneous	29.1±1.1	93.8±0.9
		8	6.8±0.1	29710±	Homogeneous	28.1±1.1	93.1±1.4
14	RT 30°C±2°C	1	6.4±0.2	23509±	Homogeneous	21±1.1	93.2±1.1
		2	6.3±0.2	39127±	Homogeneous	37.1±1.1	93.5±0.9
		5	6±0.1	52198±	Homogeneous	34.9±0.9	92.6±1.1
		8	6.2±0.2	50123±	Homogeneous	38.7±1.1	92.1±1.1
	4°C±2°C	1	6.1±0.1	33671±	Homogeneous	24.3±0.8	92.7±1.2
		2	6.2±0.1	48193±	Homogeneous	26.7±1.1	92.5±0.9
		5	6.5±0.1	49100±	Homogeneous	28±1.1	93.1±0.8
		8	6.4±0.2	52475±	Homogeneous	27.1±1.1	93.4±1.1
30	RT 30°C±2°C	1	6.4±0.2	20918±	Homogeneous	31.9±1.2	92.8±1.2
		2	6.1±0.1	37162±	Homogeneous	34.2±1.3	91.5±1.2
		5	6±0.2	39018±	Homogeneous	38.4±1.2	90.2±1.1
		8	6.5±0.2	51092±	Homogeneous	39.2±1.3	90.4±0.9
	4°C±2°C	1	6.4±0.2	38190±	Homogeneous	39.4±0.09	91.4±0.9
		2	6.8±0.1	51092±	Homogeneous	31.5±0.8	93.2±0.8
		5	6.6±0.2	50162±	Homogeneous	37.5±1.3	93.9±1.1
		8	6.3±0.1	53851±	Homogeneous	35.5±1.4	93.2±1.2
45	RT 30°C±2°C	1	6.2±0.1	29133±	Homogeneous	20.2±1.2	90.2±1.1
		2	6.1±0.1	39812±	Homogeneous	27.1±1.1	90.5±1.1
		5	6.2±0.2	42386±	Homogeneous	24.3±1.1	90.7±1.2
		8	6.8±0.2	51893±	Homogeneous	29.2±1.1	90.9±1.1
	4°C±2°C	1	6±0.2	21677±	Homogeneous	30.2±1.1	92.3±0.9
		2	6.1±0.2	25643±	Homogeneous	34.3±1.1	91.4±1.1
		5	6.8±0.2	36710±	Homogeneous	35.6±1.1	92.1±0.9
		8	6.8±0.1	48176±	Homogeneous	33.2±1.1	91.2±0.8

Parameters are derived using mean±SD. SD: Standard deviation

Table 26: Comparison of phytosomal gel with marketed formulation

Concentration (μg/ml)	Phytosomal gel (%RSA)	Marketed formulation (%RSA)
0	0	0
20	35.98	32.11
40	46.87	43.09
60	57.99	52.31
80	66.72	60.72
100	71.21	69.06

%RSA: Radical scavenging activity

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Hence, the prepared herbal antiaging phytosomal gel which is non-toxic, safe, and effective is found to be highly acceptable.

ACKNOWLEDGMENT

We would like to show our gratitude to the Verjinia CU and Dr. Deepa T Vasudevan for sharing their pearls of wisdom with us during the course of this research, and we thank the "anonymous" reviewers for their so-called insights and their comments on an earlier version of the manuscript.

AUTHOR'S CONTRIBUTIONS

Verjina CU, Deepa T Vasudevan developed and optimized the formulations. Julie Mariam Joshua and Athira Anilkumar wrote the manuscript with the support and supervision of Saritha A Surendran.

CONFLICT OF INTEREST

All authors have none to declare.

REFERENCES

1. Harman D. Aging: Overview. Ann N Y Acad Sci 2001;928:1-21.
2. Winker MA. Aging in the 21st century: A call for papers. Arch Intern Med 2002;162:745-52.
3. Sehl ME, Yates FE. Kinetics of Human Aging: I. Rates of senescence between ages 30 and 70 years in healthy people. J Gerontol 2001;56:198-208.
4. Hughes KA, Alipaz JA, Drnevich JM, Reynolds RM. A Test of Evolutionary Theories of Aging. Proceedings of the National Academy of Sciences of the United States of America; 2002. p. 14286-91.
5. Gilchrest BA. Skin aging 2003: Recent advances and current concepts. Cutis 2003;72:5-10.
6. Lapiere CM. The ageing dermis: The main cause for the appearance of old skin. Br J Dermatol 1990;122:5-11.
7. Bosset S, Barré P, Chalon A, Kurfurst R, Bonté F, André P, *et al.* Skin ageing: Clinical and histopathologic study of permanent and reducible wrinkles. Eur J Dermatol 2002;12:247-52.

8. Rojas J, Londoño C, Ciro Y. The health benefits of natural skin uva photoprotective compounds found in botanical sources. Int J Pharm Pharm Sci 2016;8:13-23.
9. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A, *et al*. Indian medicinal herbs as sources of antioxidants. Food Res Int 2008;41:1-15.
10. Kumpulainen JT, Salonen JT. Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease. Great Britain: The Royal Society of Chemistry; 1999. p. 178-87.
11. Ge L, Yong JW, Tan SN, Yang XH, Ong ES. Analysis of cytokinin nucleotides in coconut (*Cocos nucifera* L.) water using capillary zone electrophoresis-tandem mass spectrometry after solid-phase extraction. J Chromatogr A 2006;1133:322-31.
12. Squadrito GL, Pryor WA. Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite, and carbon dioxide. Free Radic Biol Med 1998;25:392-403.
13. Clark AM, Hufford CD. Discco and development of novel prototype antibiotics for opportunistic infections related to the acquired immunodeficiency syndrome. In: Human Medical Agents from Plants. Washington, DC: American Chemical Society; 1993. p. 228-41.
14. Gutteridge JM. Free radicals in disease processes: A compilation of cause and consequence. Free Rad Res Commun 1995;19:141-2.
15. Halliwell B. How to characterize an antioxidant: An update. Biochem Soc Symp 1995;61:73-101.
16. Leong LP, Shui G. An Investigation of Antioxidant capacity of fruits in Singapore markets. Food Chem 2002;76:69-75.
17. Prior RL. Fruit and vegetables in the prevention of cellular oxidative damage. Am J Clin Nutr 2003;78:570-8.
18. Devasagayam TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD, *et al.* Free radicals and antioxidants in human health: Current status and future prospects. J Assoc Physicians India 2004;52:794-804.
19. Zétola M, De Lima TC, Sonaglio D, González-Ortega G, Limberger RP, Petrovick PR, *et al.* CNS activities of liquid and spray-dried extracts from *Lippia alba* verbenaceae (Brazilian false melissa). J Ethnopharmacol 2002;82:207-15.
20. Büyükokuroğlu ME, GülçIn I, Oktay M, Küfrevioğlu OI. *In vitro* antioxidant properties of dantrolene sodium. Pharmacol Res 2001;44:491-4.
21. Shahidi F, Wanasundara PK. Phenolic antioxidants. J Food Sci Nutr 1992;32:67-103.
22. Prathapan A, Rajamohan T. Antioxidant and antithrombotic effect of tender coconut water in myocardial infarction. J Food Biochem 2011;35:1501-7.
23. Branen AL. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. J Am Oil Chem Soc 1975;52:59-63.
24. Ito N, Fukushima S, Hagiwara A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxyanisole in F344 rats. J Natl Cancer Inst 1983;70:343-52.
25. Rajamohan T, Prathapan A. Antioxidant and antithrombotic effect of tender coconut water in myocardial infarction. J Food Biochem 2011;35:1501-7.
26. Tiwari A. Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidant therapy. Curr Sci 2011;81:1179-85.
27. Joyce DA. Oxygen radicals in disease. Adv Drug React Bull 1987;127:127-476.
28. Khaing TA. Evaluation of the antifungal and antioxidant activities of the leaf extract of *Aloe vera* (*Aloe barbadensis* Miller). World Acad Sci Eng Technol 2011;75:75-76.
29. Kumar A, Kumar B, Singh SK, Kaur B, Singh S. A review on phytosomes: Novel approach for herbal phytochemicals. Asian J Pharm Clin Res 2014;10:41-7.
30. Mali RG, Hundiwale JC, Sonawane RS, Patil RN, Hatapakki BC. Evaluation of *Capparis deciduas* for anthelmintic and antimicrobial activities. Indian J Natl Prod 2004;20:10-2.
31. Chaudhary SR, Chavan MJ, Gaud RS. Anti-inflammatory and analgesic Activity of *Capparis zeylanica* root extracts. Indian J Natl Prod 2004;20:36-9.
32. Miladi S, Damak M. *In vitro* antioxidant activities of *Ale vera* leaf skin extracts. J Soc Chim Tunis 2008;10:101-9.
33. Sabhi M, Ramezanian M, Jaffari G, Haravi G, Bahaeddini F, Aynehi Y. Survey of Indian plants for Saponins, alkaloids, flavanoids and tannins, the plant of capparidaceae. Int J Crude Drug Res 1985;23:165-77.
34. Cordell GA. Introduction to the Alkaloids Biogenetic Approach. New York: John Wiley and Sons Publication; 1981. p. 892-5.
35. Bhaskar G, Arshia S, Priyadarshini SR. Formulation and evaluation of topical polyherbal anti acne gels containing *Garcinia mangostana* and *Aloe vera*. Pharm Mag 2009;5:93-9.
36. Halliwell B. Kinetin delays the onset of ageing characteristics in human fibroblasts. Biochem Biophys Res 1994;201:721-4.
37. Nair SS, Majeed S, Sankar S, Jeejamol, Mathew M. Formulation of some antioxidant herbal creams. Hygeia 2009;1:44-6.
38. Prior RL. Formulation of antioxidant gels. Am J Clin Nutr 2003;1(3):570-1.
39. Zibbu G, Batra A. *In vitro* and *in vivo* determination of phenolic contents and antioxidant activity of desert plants of *Apocynaceae* family. Asian J Pharm Clin Res 2012;5:76.
40. Dua D, Srivastava NS. Study on antioxidant and anti-aging properties of few medicinal plants. Int J Pharm Pharm Sci 2016;8:344-7.
41. Sasidharan S, Jothy SL, Zuraini Z. Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitory activities of *Cassia fistula* seeds extract. J Med Plants Res 2011;5:1941-7.
42. Houstis N, Rosen ED, Lander ES. Review on antioxidants. Nature 2006;440:944-8.
43. Fresquet F, Pourageaud F, Leblais V, Brandes RP, Savineau JP, Marthan R, *et al*. *In vitro* antioxidant models. Br J Pharm 2006;148:714-9.
44. Halliwell B, Gutteridge JM, Cross CE. Significance of antioxidants. J Lab Clin Med 1992;119:598-9.
45. Murtaza G, Waqas MK, Akhtar N, Ahmad M, Khan HM, Iqbal M, *et al*. Formulation and characterization of a cream containing extract of fenugreek seeds. Acta Pol Pharm Drug Res 2010;67:173-8.
46. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Bot J 1999;33:90-9.
47. Kaur C, Kapoor HC. Power of antioxidants. J Food Sci Technol 2001;36:703-4.
48. Kinsella JE, Frankel E, German B, Kanner J. Herbal drug delivery system. J Food Technol 1993;341(8843):85-9.
49. Bernatoniene J, Masteikova R, Davalgiene J, Peciura R, Gauryliene R, Bernatoniene R, *et al*. Topical application of *Calendula officinalis* (L.): Formulation and evaluation of hydrophilic cream with antioxidant activity. J Med Plants Res 2011;5:868-77.
50. Madhavi DL, Salunkhe DK. In: Madhavi DL, Deshpande SS, Salunkhe DK, editors. Antioxidant Rich Foods, Food antioxidants. New York: Dekker; 1996. p. 239-45.
51. Wadher KJ, Lakhotla CL, Umekar MJ. Formulation and evaluation of cream of *Azadirachta indica* leaves extracts on skin renewal rate. Int J Chemtech Res 2009;1:88-95.
52. Gazzani G, Papetti A, Masoolini G, Daglia M. Review on antioxidant containing foods. Food Chem 1998;46:4118-9.
53. Das S, Haldar PK, Pramanik G. Formulation and evaluation of herbal gel containing *Clerodendrum infortunatum* leaves extract. Int J PharmTech Res 2011;1:140-3.
54. Cevc G, Vierl U. Nanotechnology and the transdermal route: A state of the art review and critical appraisal. J Control Release 2010;141:277 -99.
55. Wickett RR, Visscher MO. Structure and function of the epidermal barrier. Dermatol Surg 2006;34:98-110.
56. Das K, Dang R, Machale MU. Formulation and evaluation of a novel herbal gel of stevia extract. Int J Pharm 1988;1:1-10.
57. Bhatia SC. Herbal chemistry. Int J Pharm Herbal Drugs 1998;23(1):22-33.
58. Tirtha SS, The Ayurveda Encyclopedia. Bayville, NY: Herbal Medicines, 1998. p. 33-62.
59. Gupta NK, Dixit VK. Development and evaluation of vesicular system for curcumin delivery. Arch Dermatol Res 2011;303:89-101.
60. Saurabh KV, Kesari A. Herbosome-a novel carrier for herbal drug delivery. Int J Curr Pharm Res 2011;3(3):7066-79.
61. Choubey A. Phytosome-a novel approach for herbal drug delivery. Int J Pharm Sci Res 2011;2:807-15.
62. Kumari P, Singh N, Cheriyan BP, Neelam J. Phytosome: A novel approach for phytomedicine. Int J Inst Pharm Sci 2011;1:84-93.
63. Nair AJ, Soman P, George A, Surendran SA. Formulation of *Myristica fragrans* (Nutmeg) topical gel and its *in vitro* evaluation for antinflammatory activity. Int J Pharm Technol 2016;8:11065-76.
64. Pillai AB, Nair JV, Gupta NK, Gupta S. Microemulsion-loaded hydrogel formulation of but enafine hydrochloride for improved topical delivery. Arch Dermatol Res 2015;307:625-33.
65. Thomas L, Viswanad V. Formulation and optimization of clotrimazole-loaded proniosomal gel using 3 2 factorial design. Sci Pharm 2012;80:731-48.
66. Ganesh N, Hanna C, Nair SV, Nair LS. Enzymatically cross-linked alginic-hyaluronic acid composite hydrogels as cell delivery vehicles. Int J Biol Macromol 2013;55:289-94.
67. Finkel T, Holbrook NJ. *In vitro* antioxidant studies. Nature 2000;408:239-40.
68. Nair NC, Henry AN. Analysis of Cytokinins. Flora of Tamil Nadu. Karnataka: Botanical Survey of India; 1983. p. 13-20.